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Formation of water-stable aggregates through the action of earthworms Implications from laboratory experiments

FRANK ZIEGLER and WOLFGANG ZECH

With one figure

Synopsis: *Original scientific paper*

The formation of water-stable aggregates through the action of earthworms was studied under controlled laboratory conditions. Mixtures of beech litter and artificial soil were incubated for 446 days at 20 °C and 70% WHC in the presence and absence of earthworms *Eisenia fetida*. The distribution of organic and mineral matter among different aggregate size fractions (> 2000, 2000–630, 630–200, 200–63, < 63 µm) was determined by means of an artificial rain experiment.

The result show that before incubation the organic matter of the beech litter-artificial soil mixtures was confined to the coarsest (> 2000 µm) and the mineral matter mainly to the finest (< 63 µm) fraction. Within 446 days without earthworms a moderate shift was observed of organic matter to finer and of mineral matter to coarser fractions. After incubation with *E. fetida*, however, about $^2/_3$ of total organic and mineral matter were present in 200–2000 µm water-stable aggregates, supposedly due to the production of compact faecal pellets.

Key words: Artificial soil, beech litter, earthworms, faecal pellets, water-stable aggregates.

1. Introduction

Earthworms play an important role in litter decomposition and incorporation of plant residues into the soil by their burrowing, feeding, and casting activities. This topic has been reviewed comprehensively by EDWARDS & LOFTY (1977) and LEE (1985). The worm casts excreted in the soil or on the soil surface, which are characterized by an intense mixing of organic matter and mineral particles, have often been described to contain more water-stable aggregates than the corresponding soil (during the last decade e.g. DE VLEESCHAUWER & LAL, 1981; LAL & AKINREMI, 1983; MACKAY & KLADIVKO, 1985; MULONGOY & BEDORET, 1989). Rather few investigations have been carried out under controlled laboratory conditions in order to quantify the process of aggregate formation from fresh organic matter and unstructured mineral soil material by earthworms. Therefore, we decided to perform model experiments of this kind in our own laboratory, and in the present study we provide information about the aggregating effect of *Eisenia fetida* on beech litter-artificial soil mixtures observed within 446 days of incubation. *E. fetida* had seen selected as the model organism since it was reasonable to start first experiments with an earthworm species that is both highly active and easy to handle.

2. Materials and methods

2.1. Incubation of beech litter with artificial soil

Twentyfive (25) g of beech leaf litter (*Fagus sylvatica* L.) and 50 g of artificial soil were placed in 800 ml plastic flower pots (10 replicates), mixed gently with a spatula, and incubated for a 446 day-period in the laboratory at 20 °C and 70% of maximum water holding capacity (WHC). The

Table 1. Physico-chemical characteristics of the artificial soil.

	coarse	medium	fine	total
sand	0%	3%	8%	11%
silt	35%	23%	12%	70%
clay				19%
*p*H			8.2	
effective cation exchange capacity			25.3 cmol(+) kg^{-1}	
organic carbon			not detectable	
total nitrogen			not detectable	

litter material was sampled in spring (Lv layer), when it had lost 95% of its initial amount of water-soluble phenolic compounds, in order to improve palatability to litter feeding animals. The artificial soil was composed of 60% powdered basalt (Kalk und Düngemittel, Langerwehe/Heistern, F.R.G.), 24% Ca-bentonite (Calcigel; Süd-Chemie AG, München, F.R.G.), 10% quartz sand (∅ 0.2–0.8 mm; Merck, Darmstadt, F.R.G.), and 6% $CaCO_3$ (precipitated; Merck, Darmstadt, F.R.G.). Physico-chemical characteristics of the artificial soil are given in table 1.

To half of the experimental pots (5 replicates) earthworms were added. For this purpose small adult and large juvenile individuals of *Eisenia fetida* (SAV.) were collected by hand from a compost heap dominated by beech leaf litter, transported to the laboratory, and left for a few days in the original compost substrate. Then the earthworms were kept overnight on moist filter paper to (at least partly) void their guts, washed in deionized water, blotted dry, and weighed. On an average 10 individuals (2.5 g biomass) of *E. fetida* were placed in the pots.

The water contents of beech litter-artificial soil mixtures were controlled by weighing and corrected on a weekly basis. After removal of the earthworms at the end of the 446 day-incubation period the contents of the pots were air-dried at 20 °C for 2 weeks. Subsequently, they were studied morphologically and subjected to chemical characterization.

2.2 Analytical methods

For chemical analyses aliquots of the air-dry samples were ground < 0.5 mm in a sieve mill. *p*H values were measured in a 1 : 10 suspension with deionized water, ash contents were obtained on ignition at 560 °C overnight. Organic carbon was analyzed by dry combustion with a Wösthoff Carmhomat 8 ADG and total nitrogen by the Kjeldahl procedure. The particle size distribution of the artificial soil was determined as described by DE LEENHEER *et al.* (1955) and the effective cation exchange capacity by use of an unbuffered 0.1 N $BaCl_2$ solution (modified according to *Mehlich*, 1948).

2.3. Artificial rain experiment

For each the treatment with and without earthworms *E. fetida* the 5 replicate beech litter-artificial soil mixtures were pooled into one mixed sample and analyzed for their actual aggregate size distribution by subjection to "artificial rain". In this experiment the samples were placed on a set of analytical sieves (mesh sizes 2000, 630, 200, and 63 µm) and irrigated uniformly by means of a sprinkling bottle. The starting material was treated the same way. As a total 300 ml of deionized water were applied per sample within a period of 1 hour. The fractions obtained by this procedure were rinsed off the sieves, collected, and air-dried at 20 °C for 2 weeks. Finally, the distribution of organic and mineral matter among the different aggregate size fractions was determined.

3. Results and discussion

Incubation of beech litter with artificial soil at 20 °C and 70% WHC led to a mass loss of about 50% within 446 days irrespective of the presence or absence of earthworms *E. fetida*

Table 2. Chemical characteristics of the starting material and the beech litter-artificial soil mixtures after 446 days of incubation in the presence and absence of *E. fetida*.

	beech litter + artificial soil 0 days	beech litter + artificial soil 446 days	beech litter + artificial soil + earthworms 446 days
*p*H	7.2 ± 0.1	7.9 ± 0.2	7.8 ± 0.1
ash (%)	70.8 ± 0.1	82.8 ± 0.6	82.7 ± 0.4
organic carbon (%)	16.5 ± 0.3	8.9 ± 0.4	9.1 ± 0.1
total nitrogen (%)	0.37 ± 0.02	0.46 ± 0.02	0.48 ± 0.03
C-to-N-ratio	45 ± 2	19 ± 1	19 ± 1
mass loss (%)		48.7 ± 1.4	49.0 ± 1.0

(table 2). This surprising result will be discussed in detail in a separate paper (ZIEGLER & ZECH 1992) and is due to an initially positive (0–179 days) and later on negative effect (179–446 days) of earthworm action on the rate of beech litter biodegradation. Despite the high conformity in chemical characteristics (table 2) the beech litter-artificial soil mixtures of both treatments differed significantly from each other in their structural properties: the residual material obtained after incubation with *E. fetida* was found to consist mainly of stable aggregates where the mineral matter was intimately associated with organic debris, whereas the considerably less structured worm-free treatment represents a very loose "side-by-side" of mineral particles and coarse leaf fragments. Thus from a morphological point of view earthworms *E. fetida* seemed to promote the formation of stable aggregates.

The results of the artificial rain experiment support this assumption. Fig. 1 illustrates the distribution of organic and mineral matter of the beech litter-artificial soil mixtures among different aggregate size fractions. Without an incubation treatment (0 days) the organic matter (present as whole beech leaves) was restricted to the > 2000 µm fraction (97%). This fraction decreased to 76% after incubation without earthworms, due to the disruption of leaves into smaller fragments and the concomitant loss of organic matter into finer fractions, initiated by combined microbial action and physical weathering processes (e.g. wetting and drying cycles). Consequently, the drastic decrease of the > 2000 µm fraction to 27% in the presence of *E. fetida* emphasizes the great importance of earthworms in litter comminution. Relatively small proportions of 4% and 2% of total organic matter were found in the 63–200 µm and < 63 µm fractions, respectively, when *E. fetida* was involved in the biodegradation process, whereas 7% and 13% of organic matter could be detected in these fractions in the case of the worm-free treatment. An overall stimulating effect of earthworm action on litter decomposition should have caused an even stronger shift to finer fractions. The explanation can very likely be deduced from the production of faecal pellets. Since the compact casts of *E. fetida* were mainly in a diameter range of about 400 to 900 µm and rarely exceeded 1200 µm, they fell under the 630–2000 µm (and partly 200–630 µm) aggregate size class. And obviously, 630–2000 µm was the predominant fraction (47%) within the organic matter distribution of the *E. fetida* treatment; the 200–630 µm fraction still contained 20% of total organic matter.

The greatest part of the mineral matter (66%) was found in the < 63 µm fraction before incubation of beech litter with artificial soil (0 days); the fractions between 63 and 2000 µm were of little importance. As a peculiarity a relatively high amount of mineral matter could be detected in the > 2000 µm fraction. But this proportion of 26%, which obviously cannot be correct in view of the actual particle size distribution of the artificial soil (no particles greater than 630 µm, table 1), is easily explained by the fact that part of the mineral particles remained on the beech leaves lying flat on the 2000 µm screen and were not completely rinsed down the set of sieves during the artificial rain experiment. This "barrier effect" of

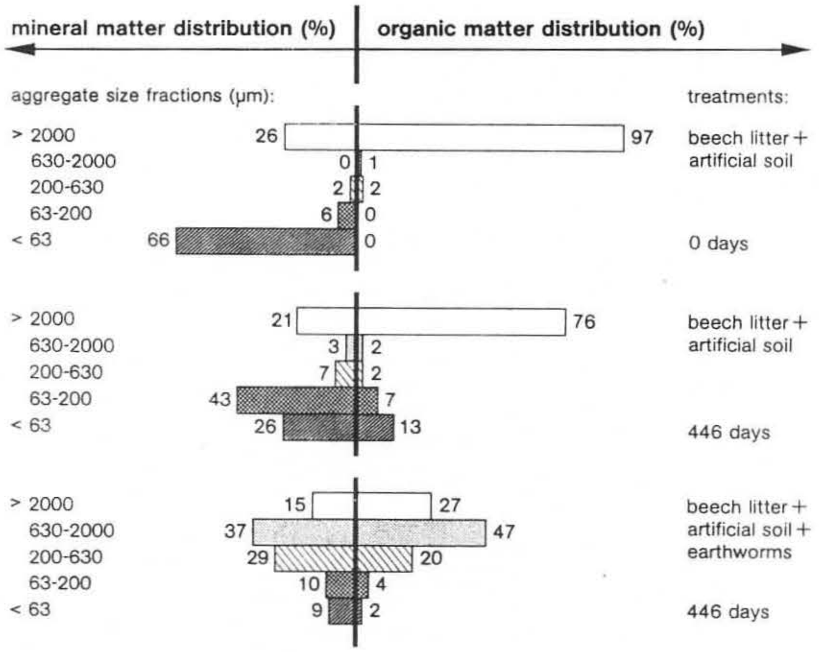

Fig. 1. Distribution of organic and mineral matter among different aggregate size fractions.

the beech leaves on the uppermost sieve diminished after 446 days of incubation as indicated by a proportion of 21% (without worms) and 15% of mineral matter (with *E. fetida*) in the > 2000 µm fraction. In the course of the 446 day-period without earthworms a shift of the mineral matter maximum occurred in favour of the 63 – 200 µm fraction, which exhibited an increase from 6% to 43%. The presence of *E. fetida* led to the formation of larger aggregates, for 29% and 37% of total mineral matter were observed in the 200 – 630 µm and 630 – 2000 µm fractions, respectively.

With regard to fig. 1 the organic and mineral matter of the beech litter-artificial soil mixtures, which initially were confined mainly to the coarsest and finest fractions, tended to meet and interact after incubation with *E. fetida* in the 200 – 2000 µm range of aggregate size, corresponding to the faecal pellets of the earthworms. Thus pelletization seems to be a very important process in the stabilization of aggregates against destruction by water. Moreover, pelletization apparently promotes also the stabilization of organic matter against decomposition by microorganisms as reflected by an earthworm-induced diminution of decay rates in advanced stages of beech litter biodegradation (Ziegler & Zech, 1992).

4. Conclusions

In the present study earthworms *E. fetida* could be shown to bind up to two thirds of total organic and mineral matter of beech litter and unstructured artificial soil in water-stable aggregates of the size range 200 – 2000 µm. This effect within a 446 day-period is considerable since *E. fetida*, which can be classified into the ecological group of épigées in the sense of Bouché (1977), prefers organic-rich materials as the natural environment. Consequently, for the endogées or anécique species living in the mineral soil an even higher stabilizing efficiency should be expected under similar experimental conditions.

5. Zusammenfassung

[Bildung wasserstabiler Aggregate durch die Tätigkeit von Regenwürmern]

Die Bildung wasserstabiler Aggregate durch die Tätigkeit von Regenwürmern wurde unter kontrollierten Bedingungen im Labor untersucht. Es erfolgte die Inkubation von Gemengen aus Buchenstreu und künstlichem Bodenmaterial für 446 Tage bei 20 °C und 70% Wasserkapazität in Gegenwart und Abwesenheit von Regenwürmern *Eisenia fetida*. Die Verteilung der organischen und mineralischen Substanz auf unterschiedliche Aggregatgrößenfraktionen (> 2000, 2000 – 630, 630 – 200, 200 – 63, < 63 µm) wurde über ein Beregnungsexperiment ermittelt.

Die Ergebnisse zeigen, daß sich vor der Inkubation die organische Substanz der Buchenstreu-Boden-Gemenge ausschließlich auf die gröbste (> 2000 µm) und die mineralische Substanz im wesentlichen auf die feinste (< 63 µm) Fraktion beschränkte. Nach 446 Tagen ohne Regenwurm-Einfluß war nur eine mäßig stark ausgeprägte Umverteilung der organischen Substanz hin zu feineren und der mineralischen Substanz hin zu gröberen Fraktionen zu beobachten. Nach Inkubation mit *E. fetida* lagen dagegen $^2/_3$ der gesamten organischen und mineralischen Substanz in wasserstabilen Aggregaten der Größe 200 – 2000 µm vor. Hierfür werden vor allem die kompakten Exkrementballen der Tiere verantwortlich gemacht.

6. Résumé

[Formation d'agrégats hydrostables par l'action des vers de terre]

La formation d'agrégats hydrostables par l'action des vers de terre a été étudiée en laboratoire en conditions controlées. Un mélange composé de litière de hêtre et de sol artificiel a été incubé pendant 446 jours à 20 °C et à 70% de sa capacité de retention en eau, ceci sans ou avec la présence de *Eisenia fetida*. La distribution de la matière organique ou minérale à différentes classes de taille d'agrégats (> 2000, 2000 – 630, 630 – 200, 200 – 63, < 63 µm) a été déterminée à l'aide d'une expérience de précipitation artificielle.

Les résultats montrent qu'avant l'incubation la matière organique de ce mélange était exclusivement sous la forme d'agrégats de grande taille (> 2000 µm) tandis que le sol artificiel correspondait essentiellement à la fraction la plus fine (< 63 µm). Après l'incubation sans l'influence des vers de terre, il n'y avait qu'une faible modification de la matière organique vers des fractions plus fines et du substrat minéral vers des fractions plus grosses. Mais après 446 jours avec *E. fetida* les $^2/_3$ de la matière organique et minérale était sous la forme d'agrégats hydrostables de taille comprise entre 200 et 2000 µm qui se dérivent probablement des turricules compactes produites par des vers de terre.

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Address of the corresponding author: Dr. F. ZIEGLER, Lehrstuhl für Bodenkunde und Bodengeographie, Universität Bayreuth, Postfach 101251, Bayreuth, D(W)-8580 (Germany).